84 Characterisation of fibrinogen degradation by the CF-related pathogens, *Burkholderia cenocepacia* and *Pseudomonas aeruginosa*

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Bacterial proteinases have been implicated in causing necrotic or hemorrhagic tissue damage. We investigated the ability of secreted bacterial proteinases, from two pathogens involved in chronic bacterial infections in cystic fibrosis, to degrade fibrinogen. We found that multiple proteinase species secreted by *Burkholderia cenocepacia* and *Pseudomonas aeruginosa* were able to degrade fibrinogen, and the degradation profile was different from that observed when fibrinogen was incubated with thrombin. Furthermore, we investigated the effect incorporation of broad-spectrum and specific proteinase inhibitors would have on the ability of bacterial proteinases to degrade fibrinogen. Treatment of *B. cenocepacia*-fibrinogen co-cultures with the individual inhibitors resulted in a partial inhibition of degradation. However, *P. aeruginosa*-fibrinogen co-cultures showed no differences in degradation profile when inhibitors were used singly. For both cultures total degradation inhibition was observed when the samples were treated with different inhibitor combinations. These results indicate that multiple bacterial proteinase species may play a role in the processes leading to the degradation of fibrinogen, which could impair polymerisation of fibrin, thus contributing to the excessive hemorrhagic tissue damage seen in the late stages of cystic fibrosis lung disease.

85* Plant host and sugar alcohol induced exopolysaccharide biosynthesis in the *Burkholderia cepacia* complex

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Burkholderia cepacia complex (Bcc) have multiple roles including soil and water saprophytes, bioremediators, plant growth promoters and plant, animal and human pathogens. As the first evidence of Bcc pathogenicity related to onion rot, we investigated growth on onion tissue to determine the "natural phenotype" of these organisms. Many Bcc isolates, which had previously been considered to be nonmucoid, produce copious amounts of exopolysaccharide (EPS) when onion tissue is provided as the sole nutrient. EPS production on onion agar was observed in all nine Bcc species investigated, but was strain-specific. Interestingly, the well-characterised ET12 strains *B. cenocepacia* J2315, K56–2 and BC7 did not produce EPS on onion agar. This finding correlated with an 11 bp deletion in the *bceB* gene encoding a glycosyltransferase which catalyses the first step of the assembly of the EPS repeat unit. Ethyl acetate extraction, analytical paper chromatography, electrophoresis and HPAE-PAD suggested that the onion components responsible for EPS induction were primarily the carbohydrates sucrose, fructose and fructans. Additional sugars were investigated, and all alcohol sugars tested were able to induce EPS production, in particular mannitol and glucitol. EPS biosynthesis did not correlate with ability to cause maceration of onion tissue. These novel and surprising insights into EPS biosynthesis show that a potentially virulent phenotype may not be detected by routine laboratory culture. Our results also highlight a potential hazard in the use of inhaled mannitol as an osmolyte to improve mucociliary clearance in individuals with cystic fibrosis.

86* Lactate production and *Pseudomonas aeruginosa* metabolism in CF sputum

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In CF sputum *Pseudomonas aeruginosa* metabolizes anaerobically. For energy generation, the bacteria can use pyruvate produced from glucose via anaerobic glycolysis. Since pyruvate can be converted to lactate and *vice versa*, we investigated if *P. aeruginosa* may benefit from externally produced lactate. In 22 CF sputum samples, in aerobically and anaerobically grown cultures of *P. aeruginosa* and *Staphylococcus aureus*, and in neutrophils lactate was determined spectrophotometrically and gaschromatographically. After three days of anaerobic growth *P. aeruginosa* gene expression was determined using Affymetrix® microarrays. Lactate in CF sputum came to 2.9±3.1 mmol/L (range 0.2 to 14.1 mmol/L) with similar concentrations in sputum colonized with *P. aeruginosa* and *S. aureus* (3.2±3.7 *vs*. 2.4±1.5 mmol/L, $p = 0.30$). During anaerobic incubation, neutrophils produced 3.2 mmol/L and *S. aureus* 2.8 mmol/L. *P. aeruginosa* did not at all generate lactate (detection limit: 0.1 mmol/L), neither with nor without oxygen. *P. aeruginosa* [1×10^7 cfu/ml] spiked with 10 mmol/L lactate did not consume lactate. The lactate dehydrogenase genes were anaerobically downregulated (PA0927 –11fold, PA2382 –3fold) or unchanged (PA4771). The genes encoding for pyruvate decomposition to acetyl CoA were upregulated 208 fold (PA3416) and 47 fold (PA3417), respectively. We could show that *P. aeruginosa* does not benefit from externally produced lactate. We confirmed the important role of pyruvate metabolism for anaerobic *P. aeruginosa* energy generation. Whether lactate production of neutrophils or *S. aureus* contributes to CF lung pathophysiology still remains to be investigated.

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87 Strict anaerobes persist in CF sputum despite antibiotic treatment

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The oxygen partial pressure in sputum plugs in the CF lung is extremely low. Besides facultatively anaerobic bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* also strict anaerobes are found in most CF patients. We analyzed antibiotic susceptibilities and bacterial counts of strict anaerobes before and after antibiotic treatment.

In sputum samples from 24 CF patients (age 29.0±9.5 yrs) strictly anaerobic bacteria were identified using an anaerobic bench and the RapID AnaII® and Vitek® identification systems. E-test® susceptibility testing was performed. In 8 patients anaerobes were counted before and after antibiotic treatment (all patients received inhaled tobramycin and/or colistin plus oral acithromycin, 6 patients received additional i.v.-courses).

In 36 anaerobe strains, meropenem was most effective (89%) followed by piperacillin/tazobactam (75%), clindamycin (69%), ceftazidim (47%), and metronidazol (42%). Anaerobe numbers remained similar before and after therapy ($2.3 \times 10^7 \pm 3.5 \times 10^8$ vs. $6.5 \times 10^6 \pm 1.4 \times 10^8$, $p = 0.21$). Also the facultative anaerobes did not change significantly ($6.7 \times 10^7 \pm 8.2 \times 10^8$ vs. $3.3 \times 10^7 \pm 4.5 \times 10^8$, $p = 0.33$). Despite in vitro susceptibility strict anaerobes – similar to the facultative anaerobes – cannot be eradicated in CF sputum. High prevalence, high bacterial counts in sputum, and persistence despite therapy suggest involvement of strict anaerobes in CF lung pathogenicity. Possibly CF patients without strictly anaerobic lung colonization may suffer from less severe disease.